Asian Journal of Agricultural and Horticultural Research

Volume 11, Issue 1, Page 15-19, 2024; Article no.AJAHR.111180
ISSN: 2581-4478

Isolation and Inoculation Effect of *Trichoderma reesei* on Growth and Yield of Barley

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2024/v11i1299

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111180

Original Research Article

Received: 02/11/2023
Accepted: 05/01/2024
Published: 07/01/2024

ABSTRACT

In today's world, usage of chemical fertilizers has become necessity for betterment of crop yield; however they have negative impact on the environment, quality of soil and human health. Therefore, involvement of substitute for chemical fertilizers is an essential requirement of present time. Plant growth promoting fungi (PGPF) serves as a best alternative in place of chemicals to enhance plant growth, crop productivity and improve nutrients availability for plants. They also show an involvement in ceasing growth of plant pathogens, hence acting as a bio control agent. The aim of our study is to screen and examine plant growth promoting fungi effect on barley crop. A total seven fungal strains were isolated from wheat rhizosphere. The isolated fungal strains were screened for their *In vitro* plant growth promotional traits. Among total, one isolate 14F found positive for five different plant growth promoting traits. This isolate was further identified at molecular level by amplification and sequencing of ITS gene region and was identified as *Trichoderma reesei*. *Trichoderma reesei* was inoculated with barley seeds and its effects were

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analyzed. Significant increase was observed in terms of plant height (root length and shoot length), plant weight (Dry and wet weight) as compared to un-inoculated plants. This *Trichoderma* strain could become a fantastic bio-fertilizer for sustainable agriculture.

Keywords: Chemical fertilizers; plant growth promoting fungi; barley; sustainable agriculture.

1. INTRODUCTION

The demands for use agro-chemicals in agricultural practices have elevated in order to meet the increasing food demands. The agrochemicals include pesticides, chemical fertilizers and plant growth promoters [1]. These chemicals play an important role by making nutrients available to the plant and protecting crops from biotic and abiotic stresses. However, the excessive use of agro chemicals has an adverse effect on the on environment. In order to protect the crops from such deleterious effects, the application of microorganisms possessing plant growth promoting properties are involved [2]. Plant growth promoting fungi are non sporulating organisms including species *Trichoderma, Fusarium, Aspergillus* and *Phoma* which are most widely distributed species. *Trichoderma* is filamentous fungi which are found in rhizospheric soil, belonging to the family of Hypocreaceae. Approximately, 300 molecularly characterized species are reported to be included in genus Hypocrea/*Trichoderma* [3]. *Trichoderma* sp. acts as biocontrol agents protecting the crops from the attack of plant pathogens and other environmental stresses. This species not only protect the pathogenic attack but also leads to plant growth promotion and seed germination. Nearly, 20 species related to genus *Trichoderma* are considered to have biocontrol properties which include species, *Trichoderma viride, T. harzianum*, *T. hamatum* and *T.reesei* [4]. Among *Trichoderma* species, *Trichoderma reesei* is chiefly studied with wide ranging applications in the field of science [5-6]. *Trichoderma reesei* is a filamentous fungus belongs to family Hypocreaceae and is important specie known for its ability to promote the plant growth as well as acting as biocontrol agent against various plant pathogens. Ultimately, application of *Trichoderma reesei* sp. is an environment friendly approach of improving production of crop. Therefore, the aim of our study was to screen, characterize and observe the effect of *Trichoderma* on the growth and development of Barley plant for sustainable agriculture.

2. METHODOLOGY

2.1 Soil Sample Collection

The rhizospheric soil samples were collected from wheat rhizosphere from agricultural fields of the nearby areas of Chandigarh lies between coordinates of 30.7688° N, 76.5746° E. The collected samples were transferred to laboratory for further investigation.

2.2 Fungal Isolation from the Soil Samples

The fungal isolates collected from the wheat soil sample were isolated by the method of serial dilution on Potato Dextrose Agar (PDA) medium plates. These fungi containing media plates were kept for an incubation period of 2-4 days at 28±1℃.

2.3 Fungal Identification

The fungal isolates were initially characterized based on the basis of colony morphology such as colony shape, size, color and microscopic examination. In addition, the fungal isolates were characterized by their microscopic structure *viz*., hyphae and type of sporulation.

2.4 Plant Growth Promoting Characterization

The isolates were tested for their plant growth promoting traits such as Zinc solubilizing test, Phosphate solubilizing test, hydrogen cyanide production test and Indole acetic acid production test.

2.5 Molecular Characterization

Potential fungal isolates showing multiple plant growth promoting test were further subjected to molecular identification using amplification and sequencing of ITS region of the genome. Forward and reverse primers used for the amplification of this region were 1(F) 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2 (R) 5'-GCTGCTTCTTCATCGATGC-3'.

2.6 Effect of Fungal Isolate on Growth of Barley Plant

Finally, potential fungal strain was inoculated with barley seeds to examine its effect on plant growth and development. Barley variety PL-426, a popular variety used in Punjab, was obtained from the seed distributer and was used in this experiment. Sterilized pots of 30 cm diameter were used which were filled with 4 kg of sterilized soil and sand in 3:1 ratio in which seed sowing was carried out. The pot experiment was conducted in three replications. Pots were irrigated with sterile tap water as needed.

3. RESULTS AND DISCUSSION

Chemical fertilizers have drastic effects on environment and animal health. Thus, scientific community is seriously involved in searching alternatives of chemical fertilizers. Thus, the aim of our study was to screen the fungi having plant growth promoting traits. Plant growth promoting fungi (PGPF) are microorganisms are one of the best suited alternatives to increase plant health and yield.

3.1 Physicochemical Characteristics of Soil

The physical and chemical properties of rhizospheric soil were examined where pH from wheat soil sample was found alkaline in nature (pH>7.5) and soil electrical conductivity (EC) was recorded 29mS/m. The total percentage of organic matter content in soil value was 0.69%, while organic carbon (C) was recorded as 0.39% and total nitrogen (N) was found 0.029% (Table 1).

3.2 Physical and Microscopic Examination of Isolates

The colonies of fungal isolates were examined on the basis of size, shape and color of colonies. Morphologically and microscopically 3 isolates (P1S1, P2S1, 14F) showing cottony white, circular shaped colonies and microscopically as branched, aseptate hyphae were identified as Trichoderma species. Similarly, 2 isolates (17F and N1K1) exhibited colony characteristics features as brown, powdery colonies and appeared as smooth colored conidiophores were identified as *Aspergillus* species. Another isolate (3F) showed cottony-greenish, irregular colonies as well as branched structure with conidia under microscope examination and was identified as *Penicillium* specie. The remaining 1 isolate (11F) revealed a structure as cottony, irregular colonies, in addition this showed aseptate hyphae with tapering conidiophores were seen under light microscopic and was identified as *Fusarium* respectively.

3.3 Plant Growth Promoting (PGP) Traits Assay of Isolates

The fungal strains were tested for the plant growth promoting traits, in which out of seven, only one (strain 14F) was tested positive for all the PGP tests *viz*. IAA Production, HCN production, Zinc solubilizing test, phosphate solubilizing test and siderophore production tests (Table 2). Our study is in agreement with many researchers concluded that *Trichoderma spp*. possesses different PGP traits [7-9].

Table1. Physicochemical properties of wheat rhizospheric soil

S.N.	Sample	pH	Electrical conductivity	Organic Carbon (%)	Organic Matter (%)	Total Nitrogen (%)
1.	Wheat rhizosphere	7.5	0.29	0.39	0.69	0.029

Table 2. *In vitro* plant growth promoting test

Isolate	IAA production (mg/ml)	HCN production	Siderophore production	Zinc solubilizing test	Phosphate solubilizing test
14F	154.6	+	+	+	+

Table 3. Effect of *T. reesei* inoculation on barley plant

Treatment	Plant height (cm)	Shoot dry weight (gm)	Root dry weight (gm)	Seeds/plant
Control	54.4 ±1.4	2.11 ±0.20	0.78 ±1.1	31
14F	71.33±2.3	4.02 ±1.2	1.6 ±1.7	76

3.4 Molecular Characterization of Potential Fungal Strain

Furthermore, molecular characterization of isolate 14F was done at species level. The retrieved fungal sequences were evaluated using a $BLAST_N$ search against the NCBI database to determine the degree of similarity. Strain 14F was identified as *Trichoderma reesei* on the basis of ITS region sequences with 99.9% sequence identity. The ITS gene sequence was submitted to NCBI GenBank Database and accession number provided was OQ581865.

3.5 Effect of Inoculation of *Trichoderma reesei* on Barley Plant

The inoculation of the isolated strain *Trichoderma reesei* was done on barley seeds of variety PL-426. Treatment of barley plants with selected isolate showed positive results in terms of overall growth and development of plant such as plant height, dry weight of plant as well as number of seeds. Plant height was measured 71.33±2.3cm, which was significantly high as compared to control plant (54.4 ±1.4). Similarly, dry weights of root and shoot also recorded higher in comparison to un-inoculated control plants (Table 3). Subsequently, plants treated with 14F strains produced higher amount of seeds (76/plant) as compared to control (31/plant). Gupta and co-workers [10] also revealed that inoculation of *Trichoderma* enhances the growth related parameters and yield of barley crop. Many researchers recorded increased crop yield after inoculation of potential biofertilizers [11-13].

4. CONCLUSION

In the present study, one potential fungal strain was isolated from the wheat rhizosphere and assessed for its plant growth promoting traits. Based on morphological and molecular examinations, the isolate was identified as *Trichoderma reesei*. On inoculation of the selected fungal isolate to specific barley plant variety, plant growth was observed in terms of plant height and weight. Additionally, the inoculated plant showed an increase in number of seeds per plant as compared to un-inoculated plants. However, in order to determine the potential of this fungal isolate, open agricultural field trials are required.

ACKNOWLEDGEMENT

The authors are thankful to Department of Biosciences, Chandigarh University, Punjab for providing all the instrumentation facilities and chemicals as well as motivating us to carry on this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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